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In vivo effects of diadenosine polyphosphates on rat renal microcirculation

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Background. Diadenosine polyphosphates (APXA) are vasoactive nucleotides that elicit effects via purinoceptors. Recent data suggest differential effects of APXA on kidney vasculature.

Methods. The in vivo effects of AP3A, AP5A, and adenosine on renal microvessels and the role of purinoceptors were investigated by the application of agonists to the hydronephrotic rat kidney and preincubation with respective antagonists.

Results. The addition of the agonists (10^{-7} mol/L up to 10^{-4} mol/L) resulted in a concentration-dependent transient vasoconstriction [interlobular artery (ILOB): adenosine 30 ± 7%, $N = 7$, AP3A 35 ± 10%, $N = 5$; AP5A 66 ± 19%, $N = 5$; 10^{-5} mol/L each] lasting up to one minute, followed by a concentration-dependent vasodilation (ILOB: adenosine 10 ± 3%, $N = 6$; AP3A 19 ± 4%, $N = 5$; AP5A 12 ± 5%, $N = 6$; 10^{-5} mol/L each). In ILOB and in the afferent arteriole (AFF), the constrictory effects of AP5A were more pronounced than those of AP3A and adenosine. In the efferent arteriole (EFF), vascular tone was only slightly affected by all agonists. The dilatory potency was comparable for all agonists in ILOB and EFF. No significant vasodilation occurred in AFF. The application of the selective A_1 receptor antagonist DPCPX (10^{-5} mol/L) completely abolished the adenosine-induced vasoconstriction, whereas the A_2 receptor antagonist DMPX and the P2 purinoceptor antagonists PPADS and A3P5P (all 10^{-5} mol/L) did not affect adenosine-induced constriction. The AP3A-induced constriction was abolished by DPCPX and was partially inhibited by PPADS. The constriction induced by AP5A was less sensitive to DPCPX but more sensitive to PPADS. In ILOB and EFF, DMPX or A3P5P abolished dilation after the addition of the agonists. The dilation after AP5A was not significantly reduced. In AFF, no significant dilation was observed with these agonists alone, but it was clearly visible in the presence of DPCPX or PPADS.

Conclusions. APXA evoke transient constrictions in vessels of the hydronephrotic rat kidney, which are mediated by A_1 and P2 purinoceptors. The length of the phosphate chain determines the degree of vasoconstriction and the extent to which the substances exert effects on the P2 purinoceptor subtypes. ILOB and AFF are more potently affected by APXA than EFF. Afferent vasodilation is partially overridden by sustained vasoconstriction.

Key words: split hydronephrotic kidney model, adenosine receptors, purinoceptors, renal microvessels, vasoactive nucleotides, hemodynamics.

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Diadenosine polyphosphates (APXA) are vasoactive purine nucleotides consisting of two adenosine molecules linked by a variable number of phosphate groups. APXAs have been shown to be present in different cell types and to be released from thrombocytes [1–3], as well as coreleased with catecholamines and adenosine 5′-triphosphate (ATP) from bovine adrenal medulla [4, 5]. Candidate metabolites of APXA are ATP, adenosine diphosphate (ADP), 3′,5′-adenosine monophosphate (AMP), and adenosine [6], which has been claimed to be the mediator of the tubuloglomerular feedback by constricting the afferent arteriole (AFF) [7, 8].

APXA have been demonstrated to differentially affect regional perfusion and to influence cardiac output and blood pressure [9], as well as the reactivity of isolated blood vessels and vascular beds [10–12]. Furthermore, the vascular effects of APXA seem to vary with the number of phosphate groups linking the adenosine molecules [13]. The influence of APXA on central and peripheral hemodynamics is currently discussed. Recent data suggest differential effects of diadenosine polyphosphates on kidney vasculature [9, 14–16].

Renal vascular effects induced by APXA and their metabolites in several models have been reported to be mediated by A_1 and A_2 adenosine receptors as well as P2 purinoceptors [7, 14–23].

We studied the effects of different diadenosine polyphosphates (AP3A and AP5A) and of the reference substance adenosine on renal microvessels in vivo. The model of the split hydronephrotic kidney used in the present study allows the examination of the microcirculation in almost all segments of the renal vascular tree in

vivo [24]. Its validity has been well established by the results in this model compared with those obtained by other approaches for a variety of vasoactive agents [25]. The natural tone of the vessels provides an opportunity to study vasodilation without having to exogenously induce preconstriction in this in vivo approach. The possibility of topical application of drugs prevents systemic effects of the compounds under investigation. The roles of A_1 and A_2 adenosine receptors as well as P2 purinoceptors in APXA-induced changes were investigated by comparing the effects of these substances on renal microcirculation before and after the addition of their respective receptor antagonists.

METHODS

Preparation of the hydronephrotic kidney

The experiments were performed on 43 female Wistar rats weighing 250 to 270 g and were in accordance with the national animal protection guidelines. The technique and experimental procedures have been described before in detail [24, 26]. Unilateral surgical hydronephrosis was induced by permanently ligating the left ureter during ketamine (Ketamine®, 10% 100 mg/kg body wt intraperitoneally; cp-pharma, Burgdorf, Germany) and xylazine anesthesia (Rompun®, 10 mg/kg body wt intraperitoneally; Bayer, Leverkusen, Germany). Two to three months later, the experiments were conducted under thiobarbital anesthesia (Inactin®, 100 mg/kg intraperitoneally; Byk Gulden, Konstanz, Germany). The rats were placed on a heated operating table to maintain rectal temperature at 37°C. The trachea was intubated, and the left jugular vein was cannulated for the continuous infusion of saline (50 μL/min), as well as for the infusion of drugs. A polyethylene catheter was inserted into the left femoral artery for continuous measurement of systemic blood pressure. The left hydronephrotic kidney was exposed by a flank incision and split along the great curvature using a thermal cautery. The dorsal half of the split kidney was sutured to a semicircular-shaped wire frame and fixed to the bottom of a Plexiglas chamber for intravital transillumination microscopy. The entry of the renal hilus into the chamber was sealed with silicon grease, and the chamber was filled with 50 mL of an isotonic, isocolloidal solution (Haemaccel®; Behringwerke, Marburg/Lahn, Germany) maintained at 37°C with a feedback-controlled heating system. The table was then mounted on the microscope stage; images of microvessels obtained through a water-immersion objective (Leitz Ultropack, UO-55; Leitz, Wetzlar, Germany) were displayed on a calibrated monitor by a closed-circuit television system and were recorded on videotape. The kidney preparation was allowed to equilibrate in the tissue bath for one hour before starting the experiments, and the stability of the preparation was tested by repeating measurements of blood pressure, heart rate, and diameters of the renal vessels at 15 minute intervals. The stability of the preparations has been demonstrated for a duration of three hours [27].

Table 1. Purinoceptors and the respective antagonists

Antagonist	DPCPX	DMPX	PPADS	A3P5P
Receptor	A_1	A_2	P2	
			P2X	P2Y
Reference	[28]	[29, 30]	[31]	[32, 33]

Renal vascular segments and vessel diameter assessment

Vessels were identified according to their branching pattern from a selected glomerulus. Lumen diameters of the following segments were measured: (*1*) interlobular artery (ILOB), (*2*) afferent arteriole (AFF), and (*3*) efferent arteriole (EFF; within 50 μm from the glomerulus). Two different kinds of description of the data were used. The means of diameters measured at the respective time in five up to eight animals form the time-diameter curves. For comparison of the three agonists without admixture or in presence of the antagonists, the respective maximal values for constriction and dilation of the individual time-diameter curves in each experiment were used.

Experimental protocols

Each protocol consisted of 12 periods of five minutes each. The diameter of the vessel in focus was measured each 4 seconds in the first minute, every 10 seconds in the second minute, and at 4 and 5 minutes. To ascertain a stable baseline and to assess eventual changes of the vessel diameter over the time even without the addition of exogenous substances, the first five-minute period served as the control. Visualizing the interlobular artery, a cumulative concentration-response curve for adenosine, AP3A, or AP5A (10^{-6}, 10^{-5}, 10^{-4} mol/L; Sigma, Deisenhofen, Germany) was obtained (periods 2 through 4). After assessing each concentration-response curve, the bathing medium was exchanged for control medium, and after an equilibration time of 10 minutes, the vessel diameter was measured three times. From these values, the mean was determined, which served as a new baseline for the following concentration-response curve. In period 5, the focus was set on the AFF, and the agonist was added at 10^{-5} mol/L. Adding the agonist at 10^{-5} mol/L in period 6, the EFF was examined. In period 7, the effects of receptor antagonists were tested (DPCPX, DMPX, PPADS, A3P5P; Sigma) the targets of which are summarized schematically in Table 1. Observing the interlobular artery, in presence of the antagonist, another cumulative concentration-response curve with an

Table 2. Interlobular artery constrictions in percent of the first vasoconstriction evoked by the same concentration of AP5A

AP5A concentration	Period														
	2	3	4	W	5	W	6	W	8	9	10	W	11	W	12
10^{-6} mol/L	100								96 ± 4						
10^{-5} mol/L		100			98 ± 1		89 ± 9			93 ± 3			95 ± 4		96 ± 6
10^{-4} mol/L			100								104 ± 16				

Periods 2 to 12 were 5 minutes each. Abbreviation is: W, washout (10 min each). Data are mean values ± SEM, $N = 3$ animals.

agonist was recorded (periods 8 through 10). Finally, the effects of this agonist at the concentration of 10^{-5} mol/L in presence of the antagonist was detected in the AFF (period 11) and EFF (period 12), respectively.

To preclude that in this in vivo model desensitization of purinoceptors occurs, we additionally performed three control experiments in three animals. For these experiments, the same protocol was used as before, except that the effect of the repetitive addition of the diadenosine polyphosphate was viewed in the interlobular artery only and that period 7, the testing of a receptor antagonist, was skipped. The results are summarized in Table 2, which provides the effects of AP5A on vessel diameter as a percentage of the change after the first addition of the substance in the respective concentration. The data show that with repeated addition, the agonists evoke the same effects. Furthermore, there were no significant differences in the duration of the interval before vasodilation started nor in the degree of the vasodilation found in the five-minute periods examined in these experiments.

Data analysis including statistical analysis

The data are presented as means ± SEM. Changes in vascular diameters are presented as a percentage of the preceding control value. The time course of mean changes in vessel diameter was processed from mean intrarenal vessel diameters ± SEM at the respective times of measurement (Fig. 1). Since peak changes in vessel diameter occurred at variable time points, all further data are derived from minimal vessel diameters during the early phase and maximal late phase diameters of the renal microvessels (Figs. 2–5). The statistical significance was determined by analysis of variance for multiple comparisons, Student-Newman-Keuls test, and paired and unpaired *t*-tests as appropriate. The overall significance level was set to $P < 0.05$.

RESULTS

Topical application of adenosine, AP3A, and AP5A to the hydronephrotic rat kidney evoked a transient constriction, which was followed by a discrete vasodilation. The peak constrictory effect developed in 10 to 20 seconds (early phase), and the vessel diameter returned to

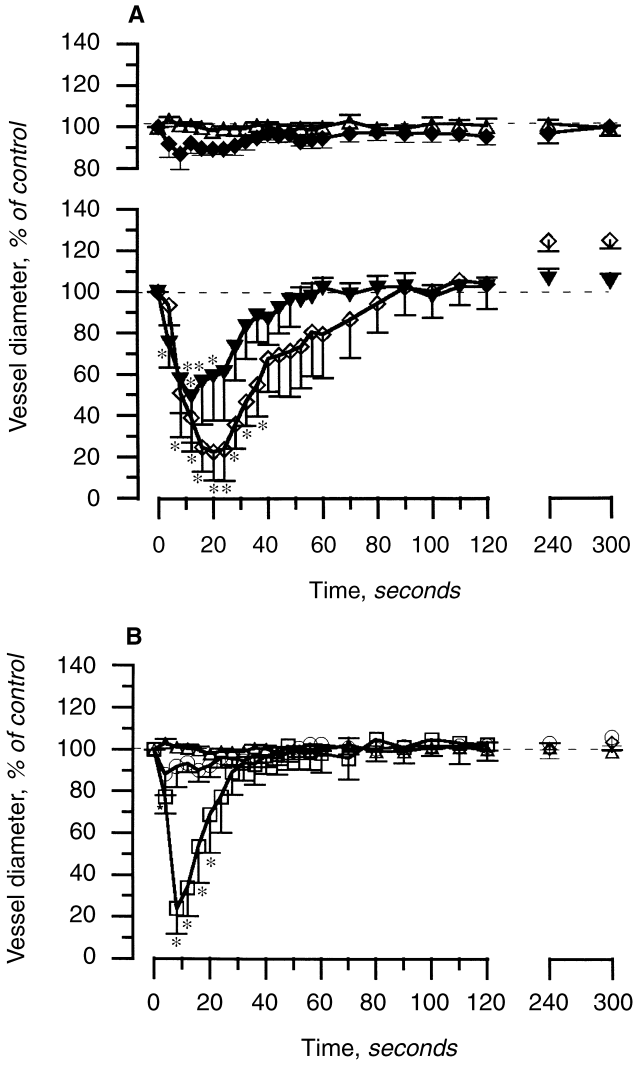

Fig. 1. Time course of mean changes in intrarenal vessel diameter after the application of AP5A. The curves show the mean intrarenal vessel diameters ± SEM at the respective times of measurement. (*A*) Effect of AP5A [10^{-6} mol/L (◆) up to 10^{-4} mol/L] on interlobular artery diameter. Only AP5A 10^{-5} mol/L (▼) and 10^{-4} mol/L (◆) showed significant constrictions in this segment. Symbol (△) is control. (*B*) Effect of AP5A (10^{-5} mol/L) on the diameters of afferent (□; AFF) and efferent (○; EFF) arterioles. AFFs were constricted significantly, while the mean EFF diameters did not change significantly. The number of animals was five to eight. *$P < 0.05$ vs. baseline.

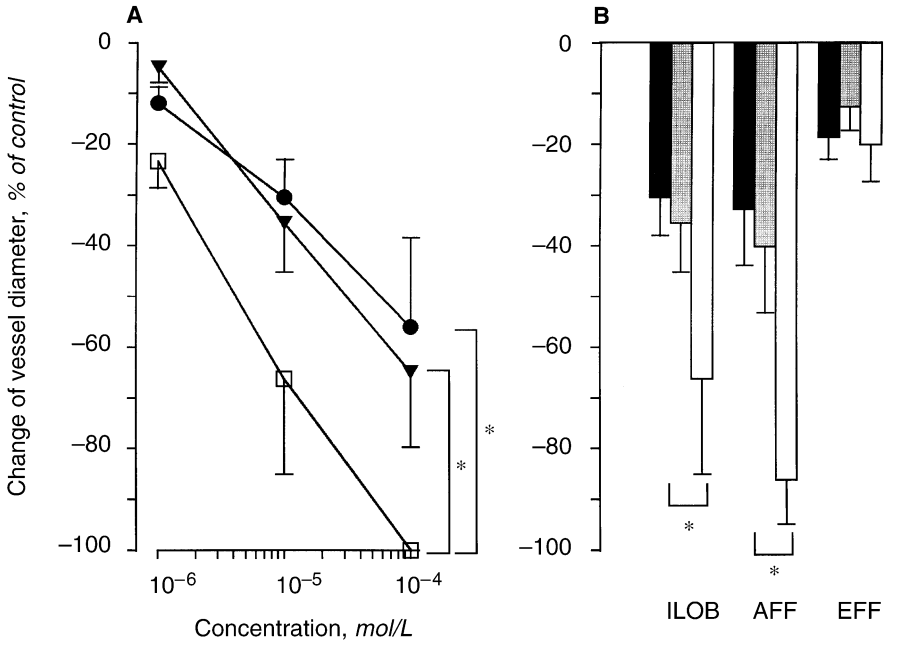

Fig. 2. Comparison of maximal constrictory responses of adenosine AP3A or AP5A on interlobular artery, afferent, and efferent arteriole diameters. (*A*) Concentration-response curves of the maximal constrictions elicited by adenosine (●), AP3A (▼), and AP5A (□) on the interlobular artery. The curve for AP5A significantly differs from those of adenosine and AP3A. (*B*) Comparison of early-phase maximal constrictions of interlobular artery, AFF, and EFF after application of adenosine (■), AP3A (▩) and AP5A (□) at a concentration of 10^{-5} mol/L each. All agonists significantly constricted every segment examined compared with baseline. The effects of AP3A and AP5A were significantly more pronounced in interlobular artery and AFF than in EFF. In both preglomerular segments, AP5A was more effective than AP3A and adenosine ($N = 5$ to 8). *Statistical significance of the difference ($P < 0.05$).

baseline levels within 1 to 2 minutes. After three minutes (late phase), vessel diameters stabilized above baseline levels. As an example of the three agonists used, Figure 1 depicts the time course surveyed after local application of AP5A (10^{-6} mol/L up to 10^{-4} mol/L) in the ILOB (Fig. 1A) and the effects of AP5A (10^{-5} mol/L) monitored in AFF and EFFs (Fig. 1B). In Figure 1, the first kind of description of the data is used. In contrast to all other graphs, the time diameter curves of Figure 1 are formed by the means of the change of vessel diameters measured at the respective time. While AP5A at the concentration of 10^{-6} mol/L did not significantly reduce the mean diameter of the ILOB at the respective time, nor did it so at 10^{-5} mol/L in the EFF, AP5A clearly induced constrictions at 10^{-5} and 10^{-4} mol/L in the ILOB and in the AFF.

To compare the three agonists without admixture or in presence of the antagonists, the means of the respective maximal changes (constriction or dilation, respectively) during the early or the late phase of the individual time-diameter curve in each experiment were used (Fig. 2–5). Adenosine, AP3A, and AP5A constricted renal microvessels concentration dependently (Fig. 2). In the ILOB, AP5A was more potent in doing so than AP3A and adenosine (Fig. 2A). Figure 2B compares the early phase minimal diameters of the ILOB of AFF and EFF after application of adenosine, AP3A, and AP5A at the concentration of 10^{-5} mol/L. The three agonists constricted all vessels significantly in comparison to baseline values. The effects were significantly more pronounced in the ILOB and the AFF than in the EFF. AP5A more potently constricted both preglomerular segments than AP3A, which was slightly more effective in doing so than adenosine.

As can be deduced from Figure 3A, in the late phase, the three agonists dilated the ILOB concentration dependently. The curves do not differ significantly, indicating a similar potency of the three agonists in dilating the ILOB. While application of adenosine and AP3A induced vasodilation in the interlobular artery (Fig. 3B), the addition of adenosine and AP3A failed to induce a significant dilation in the AFF in the five minute period examined in this study. In this interval, the AFF remained vasoconstricted after the application of AP3A. Also, in this observation period AP5A neither significantly dilated the ILOB nor AFF as a consequence of ongoing vasoconstriction. The EFF showed a slight dilation after the addition of all three agonists. Thus, inasmuch the AFF shows a long-lasting constriction after application of the agonists, the diameter changes of this last preglomerular segment differ from those patterns of interlobular artery and EFF.

To test the role of adenosine receptors and P2 purinoceptors in mediating the effects of diadenosine polyphosphates on renal microvasculature, we examined the effect of adenosine and AP3A as well as AP5A in the presence of respective receptor antagonists. Topical application of the antagonists themselves did not exert significant effects on intrarenal vessel dimensions (data not shown). Figure 4 summarizes the maximal constrictory effects of adenosine (Fig. 4A), AP3A (Fig. 4B), and AP5A (Fig. 4C) on interlobular artery without an admixture and in the presence of the A_1-antagonist DPCPX, the A_2-antagonist DMPX, or the P2-antagonists PPADS or A3P5P (10^{-5}

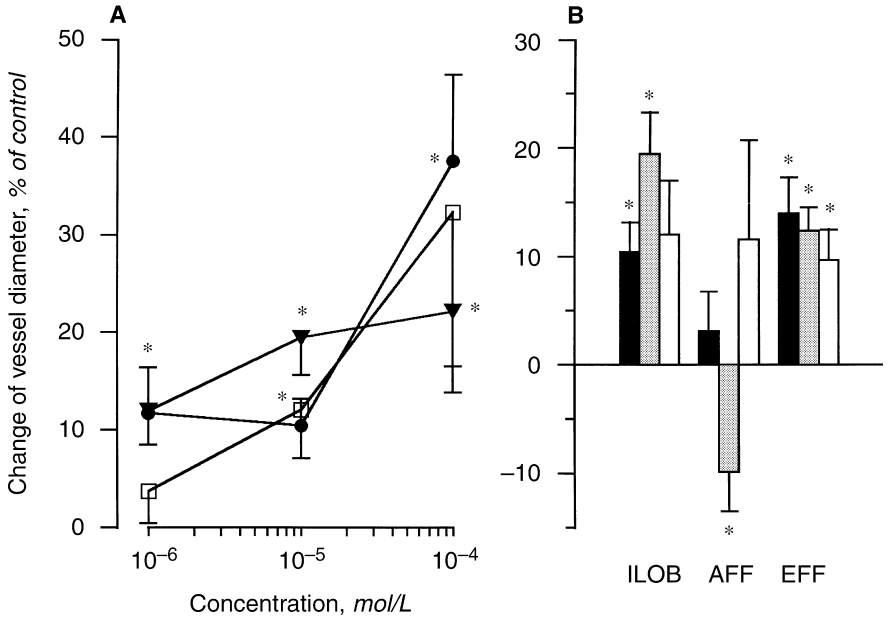

Fig. 3. Maximal dilatory effects of adenosine, AP3A and AP5A. (*A*) Concentration-response curves of the maximal dilations induced by adenosine (●), AP3A (▼) or AP5A (□) on interlobular artery (ILOB). All three agonists similarly increased the vessel diameters. (*B*) Comparison of late-phase maximal diameter changes of ILOB, AFF, and EFF after application of adenosine (■), AP3A (▩), and AP5A (□), 10^{-5} mol/L, each. In the ILOB, adenosine and AP3A and in the EFF, all antagonists increased the vessel diameters. In the AFF, no significant dilation could be observed. The number of animals was 5 to 8. *Significant difference vs. baseline ($P < 0.05$).

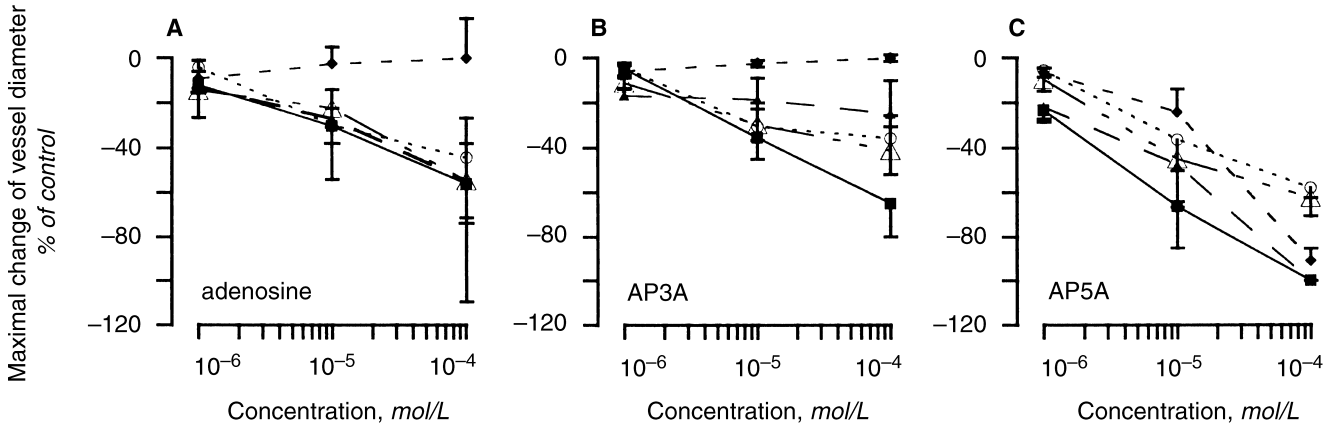

Fig. 4. Effects of DPCPX, DMPX, PPADS, and A3P5P on maximal constrictions elicited by adenosine, AP3A, or AP5A in the interlobular artery. (*A*) The effect of adenosine was significantly inhibited by DPCPX only. (*B*) The effect of AP3A was completely inhibited by DPCPX and was partially reduced by 10^{-5} mol/L DMPX, PPADS, or A3P5P. (*C*) The effect of AP5A was partially reduced by DPCPX, PPADS, or A3P5P. The number of animals was five to eight. Symbols are: (■) agonists; (◆) DPCPX; (▲) DMPX; (○) PPADS; (△) A3P5P.

mol/L, each). The concentration-dependent constriction induced by adenosine was abolished by the A_1-adenosine receptor antagonist DPCPX, whereas the A_2-adenosine receptor antagonist DMPX, as well as the P2-purinoceptor antagonists PPADS and A3P5P did not change the effect of adenosine significantly. DPCPX reduced the constrictory effect of AP3A as well, while the addition of AP3A in the presence of DMPX or PPADS was still followed by a concentration-dependent constriction, which was significantly reduced at 10^{-4} mol/L. The effects of AP5A without admixture differed significantly from those achieved in presence of DPCPX, PPADS, or A3P5P. The agonistic profile for the effects of adenosine, AP3A, and AP5A shifted from pure A_1-adenosine receptor agonism with adenosine to a more pronounced P2-purinoceptor agonism with AP5A, while the profile of AP3A was intermediate.

Figure 5 summarizes the effects of purinoceptor antagonists (10^{-5} mol/L) on late-phase vessel diameter of interlobular artery (Fig. 5A), AFF (Fig. 5B), and EFF (Fig. 5C) induced by agonists at the concentration of 10^{-5} mol/L. It can be deduced that the impairment of interlobular artery vasodilation by the A_2-antagonist DMPX and the P2-antagonist A3P5P was mirrored by the antagonist effects in AFF and EFF. The A_1-antagonist DPCPX was without any effect in the late phase in the two latter vessels. The P2-antagonist PPADS partially reduced the dilation induced by AP3A and adeno-

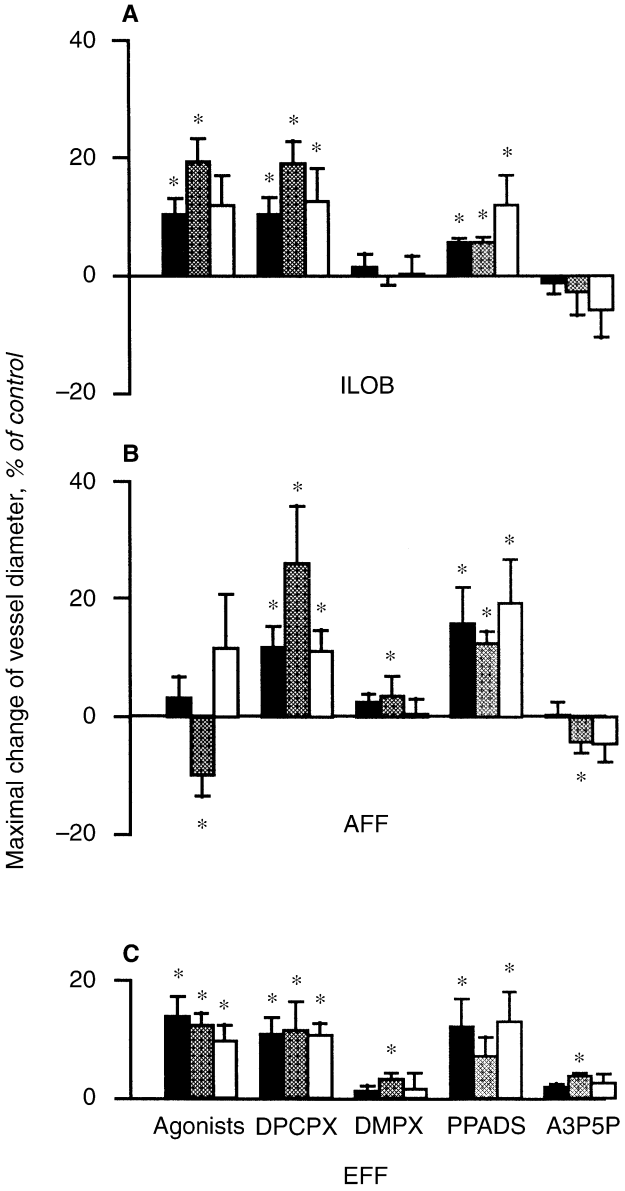

Fig. 5. Comparison of the effects of DPCPX, DMPX, PPADS, or A3P5P (10^{-5} mol/L each) on adenosine (■), AP3A (▒), or AP5A (□)-induced maximal changes in late phase vessel diameter. Effects in the interlobular artery (*A*), in the afferent (*B*), and the efferent arteriole (*C*) elicited by the agonists alone (10^{-5} mol/L, each) and in presence of the antagonists (10^{-5} mol/L, respectively). DMPX and A3P5P reduced the dilation evoked by all three agonists in the ILOB and the EFF. PPADS partially reduced the effects of adenosine and AP3A in the ILOB. AFF dilation got visible in presence of DPCPX or PPADS. The number of animals was five to eight. *Significance of the effects vs. baseline ($P < 0.05$).

sine in the interlobular artery. Interestingly, the AFF, which remained constricted or at least was not dilated significantly by the agonists, clearly showed vasodilation after application of DPCPX and PPADS. Thus, the underlying vasodilation was demasked by the addition of the A_1-adenosine receptor antagonist and the P2-purinoceptor antagonist PPADS.

Topical application of all agonists and antagonists had no effect on the heart rate or mean arterial pressure.

DISCUSSION

Renal hemodynamics are affected by diadenosine polyphosphates [9, 15, 16]. Our study evaluated their effects on intrarenal microvasculature by analyzing interlobular artery as well as afferent and efferent arteriole diameters after application of AP3A, AP5A, or adenosine (which was regarded as a reference substance) to the split hydronephrotic rat kidney. As in intact animals, various isolated vascular beds, and arteries of several species [3, 9, 12, 34], topical application of APXA induced constriction in renal microvessels of this model. As in perfused rat mesenteric arteries [13] and the isolated perfused rat kidney [15], the administration of AP5A was followed by a more intense and longer lasting constriction than admission of AP3A. In line with the higher affinity of especially AP5A and AP6A for P2X purinoceptors in ligand binding studies [35], AP5A was the more potent contractile agonist.

The fact that AP5A caused a more intense vasoconstriction than AP3A and adenosine in the preglomerular vessels, which had similar effects, contrasts with the finding of equal and less overall pronounced constrictory potencies for the EFF of the three agonists. This suggests a more intense involvement of P2 purinoceptor activation in preglomerular vasoconstriction as compared to efferent vasoconstriction. This conclusion parallels with the findings of Inscho, Mitchell, and Navar, who found that ATP, a P2 agonist, constricts AFF but not EFF, whereas adenosine constricts both vascular segments [19]. Of the major segments of the intrarenal microvasculature, the AFF is most responsive to ATP [36], which is in line with our finding that after a transient constriction caused by the addition of the agonist, AFF does not dilate unless P2X purinoceptors are blocked.

Degradation of the agonists as a cause for the transient nature of the vasoconstriction could be excluded, because the same medium that exerted a transient vasoconstriction was effective in repeatedly inducing comparable constrictions when it was collected from the bath after five minutes and added again after exchanging with control medium. Control experiments that followed the 12-period scheme demonstrate that repeated administration of APXA reproducibly constricted renal vessels. Thus, neither agonist degradation nor receptor desensitization occurred. This also suggests that desensitization is not responsible for the transient character of the effects.

Osswald reported that there was an initial reduction of renal blood flow during continuous infusion of adenosine into the renal artery, which then returned or rose slightly above preinfusion levels within one to three minutes [7]. Holz and Steinhausen found stable adenosine analogues to affect the vessel diameter continuously [37]. Here we

report transient constrictory effects of adenosine as well as of APXA. These dual effects of APXA and adenosine in microvessels of the hydronephrotic rat kidney are in line with data showing that APXA evoke a biphasic blood pressure response to APXA in anesthetized rats [9]. Interestingly, Inscho states that microvascular responses to adenosine include vasoconstriction at low doses and vasodilation at higher concentrations [18]. The vasoconstriction is elicited in both preglomerular and postglomerular arterioles. Furthermore, Inscho, Ohishi, and Navar reported arcuate and interlobular arteries to exhibit transient vasoconstrictions with ATP, while in contrast, it evoked a sustained reduction in the afferent arteriolar diameter, with the EFF remaining unchanged [36]. The fact that AP3A and AP5A, in contrast to ATP, elicit transient effects also in the AFF indicates that these agonists probably provoke purinoceptor mediated vasodilation. This is further supported by the finding that after inhibition of A_1 adenosine or P2X receptors, the AFF dilated after an addition of these agonists.

APXA have been observed to induce arterial relaxation in several vascular beds [15, 38] and in isolated agonist-constricted rat mesenteric arteries [13]. It was excluded that the relaxing responses were mediated by metabolites generated by asymmetric cleavage of the APXA [6, 10, 39]. Comparing the constrictory and dilatory potencies of adenosine and the APXA, it is evident from the present study that metabolism of APXA to adenosine does not account for the observed effects. In our system, intrarenal vessel diameters were similarly augmented by adenosine, AP5A, and AP3A in the late phase after agonist addition. The interlobular artery and EFF were dilated to a similar extent, while the AFF diameter was not significantly augmented. The latter finding may be the consequence of a more intense vasoconstriction in this segment.

Intrarenally infused P2Y agonists dilate renal vessels [40, 41]. It has been reported that by contacting the smooth muscle from the adventitial side after superfusion, no significant afferent arteriolar vasodilation occurs [21]. Released from thrombocytes, APXA may gain access to their receptors from the lumen. On the other hand, endogenous purine nucleotides are released from sympathetic nerve varicosities or in a paracrine fashion from cells neighboring the renal microvasculature. Nucleotides released from these sources are delivered to the interstitial fluid where they interact with P2-receptors on microvascular smooth muscle, mesangial cells, or tubular and glomerular epithelium. In our study, the application of the substances to the bath most likely resulted in abluminal access of the APXA to the receptors activating the smooth muscle receptors first, whereas in the isolated perfused rat kidney, compounds that are administered to the luminal side meet the endothelial cells first. This and the absence of endothelial involvement in the vasodilatory response to APXA [10, 42] render it less likely that the P2-agonists initiate the dilation by a generation of endothelium-dependent vasodilators, although rat renal purinoceptor-mediated vasodilation has been shown to be converted to vasoconstriction by nitric oxide synthase inhibition with an application of the agonist from the endothelial side [40, 41] and removal of the endothelium of intrarenal vessels converted vasodilation to constriction [41]. However, Inscho et al concluded that in superfused, blood-perfused rat juxtamedullary afferent nephrons, partial relaxation of ATP-constricted interlobular arteries and AFF was not due to endogenous nitric oxide [21].

The effects of purinoceptor antagonists indicated the role of respective receptors in mediating the effect of APXA on renal microvasculature. While there are selective antagonists for the adenosine receptor subtypes, the selectivity of PPADS and A3P5P for the P2-purinoceptor subtypes is limited (Table 1). As expected, DPCPX, the A_1-receptor antagonist, completely abolished adenosine-induced vasoconstriction, whereas the A_2-antagonist DMPX and the P2-antagonists PPADS and A3P5P had no significant effects. In the isolated perfused rat kidney, the constriction by AP3A was mostly due to A_1-receptors, that by AP4A to A_1- and P2X-receptors, and the constrictive response to AP5A was due to P2X-purinoceptor activation [15, 16]. Similarly, we found a complete inhibition of AP3A-induced vasoconstriction by the A_1-antagonist, but only partial effects of the A_2- or P2-antagonists. Instead, the vasoconstriction induced by AP5A was antagonized most effectively by the P2-receptor antagonist. Intravenous bolus administration of P2-receptor subtype agonists increase renal and mesenteric resistance and arterial pressure in rats [17]. In the present study, AP5A, in comparison to adenosine and AP3A, was less sensitive to the A_1-antagonist DPCPX but was more sensitive to the P2-antagonists PPADS and A3P5P, indicating that the vasoconstriction is mediated by A_1-receptors and P2-purinoceptors. The lack of efferent P2X-receptor expression [43] clarifies our finding that PPADS does not change the effect of adenosine, AP3A, or AP5A in this segment.

Comparable to the vasodilation evoked by AP3A in a raised tone preparation of the isolated perfused rat kidney, which was blocked by the A_2 antagonist DMPX [15], the vasodilation seen with adenosine, AP3A, and AP5A in the present study was antagonized by the A_2-antagonist DMPX and by the P2-antagonist A3P5P. Cox and Smits reported a decrease in cerebral, coronary, and mesenteric but no effects on renal vascular resistance after P2Y-receptor activation [17]. As expected, the late-phase vasodilation evoked by adenosine, AP3A, and AP5A was not altered by the A_1 antagonist DPCPX nor by the P2-antagonist PPADS. While the pattern of late-phase responses to the antagonists was similar and clear

in the interlobular artery and the EFF, it was more complex in the AFF. Vasodilation was only visible when the A_1-receptors or the P2X-receptors were antagonized. These data indicate that in the AFF, adenosine, AP3A, and AP5A induce a more intense and longer lasting constriction that overrides the underlying dilation mediated by activation of A_2-receptors or P2Y-purinoceptors.

Despite a decrease of blood flow and nearly complete lack of filtration in the hydronephrotic kidney, pathological changes of renal blood vessels are minor [24], and the model retains kidney-specific vascular properties, for example, autoregulation, response to adenosine, and natriuretic peptides that uniquely dilate preglomerular vessels and constrict EFFs [25]. Thus, the data presented herein provide relevant information on the in vivo action of diadenosine polyphosphates in renal microvessels. The results indicate that APXA and adenosine elicit dual effects on intrarenal microvessels consisting of transient vasoconstriction and subsequent discrete vasodilation. The vascular effects of the reference substance adenosine observed in the hydronephrotic kidney could be ascribed to activation of A_1- or A_2-adenosine receptors, while APXA additionally activate P2-purinoceptors. The length of the phosphate chain of APXA determines the degree of vasoconstriction and the extent to which the compounds exert effects on the P2-purinoceptor subtypes. APXA exert differential effects on the segments of intrarenal vasculature. Interlobular artery and particularly AFF are more potently affected by APXA than the EFF, whereby afferent vasodilation is overridden by intense vasoconstriction.

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APPENDIX

Abbreviations used in this article are: ADP, adenosine diphosphate; AFF, afferent artery; AMP, 3′,4′-adenosine monophosphate; APXA, diadenosine polyphosphatases; AP3A, diadenosine triphosphate; AP5A, diadenosine pentaphosphate; A3P5P, adenosine 3′,5′-bisphosphate; ATP, adenosine 5′-triphosphate; DMPX, 3,7-dimethyl-l-propargylxanthine; DPCPX, 8-cyclopentyl-3,7-dihydro-1,3-dipropyl-1H-purin-2,6-dione; EFF, efferent artery; ILOB, interlobular artery; PPADS, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid.

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